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(54) Immobilisation of polynucleotides

(57) The problem of immobilising a polynucleotide on a welled plastics support, such as a 96-well microtitre plate, has been overcome by the finding that a branched polyethylenimine bonds to the plate and can be bonded to polynucleotides, such as DNA, RNA or oligonucleotides, by a cross-linking agent such as glutaraldehyde. A polynucleotide present in an analyte can be assayed by binding it to the plate, probing it with a labelled complementary polynucleotide under hybridisation conditions, and detecting the presence or amount of the label.

The plate is made preferably from polyvinyl chloride or polystyrene.

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IMMOBILISATION OF POLYNUCLEOTIDES

Background of the invention

1. Field of the invention

This invention relates to the immobilisation of polynucleotides such as nucleic acids on rigid plastics surfaces such as welled plastics plates, especially those usually referred to as microtitre plates, or plastics tubes.

2. Description of prior art

It is known to immobilise polynucleotides, especially DNA, on nitrocellulose and diazo papers and on nylon-based membranes, and to carry out hybridisations using such immobilised DNA. It would be an advantage to be able to immobilise polynucleotides on welled plastics plates such as microtitre plates, conventionally having 8 rows of 12 wells, so that assays depending on hybridisation of the polynucleotide to a complementary polynucleotide in an analyte can readily be carried out in the wells. However, a satisfactory method of immobilising DNA on the plastics material of the plates has not so far been devised.

There is a body of literature relating to the immunoassay of native DNA, for example in a patient suffering from systemic lupus erythematosus. In such immunoassays the wells of a microtitre plate are coated with a basic protein or polypeptide, especially methylated BSA, poly-L-lysine or protamine (a protein containing a high proportion of residues of basic amino acids and obtained from fish sperm). "Antigen DNA", such as calf thymus DNA is then added, the attraction between protonated amino groups of the protein or polypeptide and phosphate groups of the DNA apparently being responsible for binding the DNA to the protein or polypeptide. The "antigen DNA" will bind to anti-DNA antibodies in the analyte and bound anti-DNA is detected, typically in the usual manner of a sandwich ELISA by adding anti-human immunoglobulin conjugated to an enzyme label, washing and assaying the bound label. See, for example M. Kavai et al., J. Immunological Methods 48, 169-175 (1982),

R. Rubin et al., ibid. 359-366 (1983), especially the discussion on pages 364 and 365, and B. Leipold and W. Perry ibid. 66, 227-234 (1984). It has been claimed that single-stranded DNA can be absorbed directly on to polystyrene tubes, for use in immunoassay, see E. Engvall, The Lancet ii, 1410 (1976).

While the above methods of binding DNA to plastics microtitre plates or tubes have been used in immunoassay, it has remained a problem how to achieve sufficiently strong binding of DNA to such plastics supports to withstand the severe conditions of hybridisation. Hybridisations are typically carried out in a buffer containing significant loadings of salts, e.g. 0.5 to 1.0 M sodium chloride and frequently a washing at a temperature well above 37°C is required to remove any second strand DNA not stringently bound (and therefore not wholly complementary) to the first strand DNA.

Y. Nagata et al., FEBS Letters 183, 379-382 (1985) have described a method of binding DNA to microtitre plates ("Microfluor", Dynatech), for use in a hybridisation assay, in which denatured DNA in a buffer containing magnesium or calcium ions was incubated overnight in the plate wells, the solution removed and the DNA fixed by UV irradiation. The paper shows only picogram amounts of DNA bound to the wells.

Further prior art is described after the Summary of the invention, without which its context would not be clear.

Summary of the invention

It has now been found that polyethylenimine (PEI), a branched polymer having free primary or cross-linkable secondary amino and also a proportion of cationic protonated amino groups, will bond well to most plastics surfaces and also, with the aid of an appropriate cross-linking agent, such as glutaraldehyde to a polynucleotide.

In an important aspect, the present invention provides a rigid plastics support, especially a welled plastics plate, having bonded thereto a polyethylenimine. Such plates can be stored for considerable periods.

The invention further provides a method of immobilising a polynucleotide on a plastics support, especially in wells of a welled plastics plate, which comprises incubating a support as defined above (having the PEI bound thereto) with the polynucleotide and with a cross-linking agent effective to link the primary amino groups of the PEI to reactive groups on the polynucleotide.

The invention enables assays for polynucleotides to be carried out. Thus, a method of assay of a polynucleotide in an analyte may comprise:

(1) if the polynucleotide is not already in a single-stranded form, free of secondary structure or duplex which would interfere with hybridisation, denaturing it to remove said secondary structure or duplex,

(2) immobilising the polynucleotide on the support, preferably in wells of a welled plastics plate, by the immobilisation method defined above,

(3) probing the immobilised polynucleotide with a labelled complementary polynucleotide under hybridisation conditions, and

(4) detecting the presence or amount of label bound to the support, i.e. to the wells where a welled plastics plate is used.

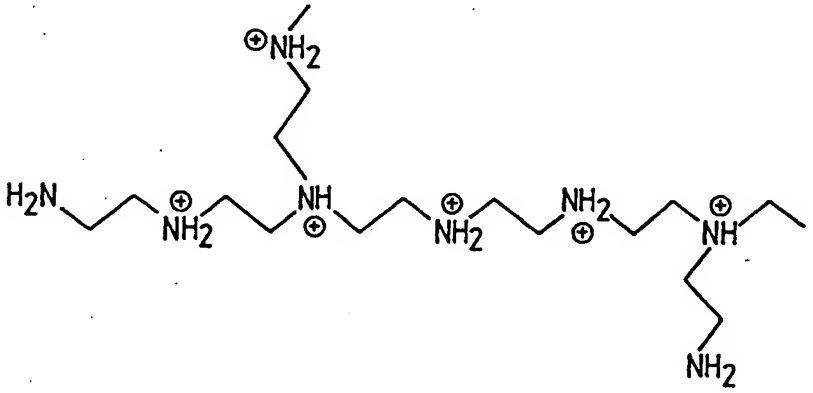
Additional description of prior art

Polyethylenimines are polymers obtainable by the ring-opening cationic polymerisation of ethylenimine of formula (1).

(1)

and are highly branched, and believed to contain primary, secondary, and tertiary amine groups in the ratio of approximately 1:2:1. There is experimental evidence that branching sites are separated mainly by secondary amine groups, with about one branch for every 3

to 3.5 nitrogen atoms within a linear chain as depicted in the illustrative, non-limiting formula (2) below



(2)

They have been reviewed by D. Horn of BASF in "IUPAC International Symposium on Polymeric Amines and Ammonium Salts, September 24-26, 1979, Ghent, Belgium", pages 333-355. This review includes on pages 341-345 a discussion of adsorption of PEI onto cellulose and silica.

M. Renz and C. Kurz, Nucleic Acids Research 12, 3435-3444 (1984) describe a method of linking a protein to DNA, whereby the protein serves as a label for the DNA. Preferably the protein is an enzyme which is readily detectable by reaction with an appropriate substrate. The linking reaction involves covalently bonding benzoquinone to horseradish peroxidase or alkaline phosphatase and then cross-linking the enzyme to DNA by use of PEI and glutaraldehyde. The DNA thus labelled is useful as a probe. The authors suggest that the PEI reacts with the benzoquinone to form a positively charged tail on the enzyme. The DNA interacts with the tail by ionic binding, presumably (although the paper does not say so) through negatively charged phosphate groups on the DNA interacting with protonated amino groups of the PEI. The authors say that this ionic binding is essential for a successful cross-linking reaction (with the glutaraldehyde) leading to covalent bonds.

W.E. Meyers et al., J. Amer. Chem. Soc. 99, 6141 and 6142 (1977) have prepared a catalyst which they describe as a "polyethylenimine ghost". PEI is adsorbed onto porous alumina beads and then cross-linked with glutaraldehyde. The imine linkages are reduced with sodium borohydride. The alumina support is then removed by leaching with 1 N hydrochloric acid. The PEI ghosts were reacted with a protected histidine derivative to introduce histidyl groups. The imidazole function of the histidyl groups acted as a catalyst for the hydrolysis of p-nitrotrifluoroacetanilide.

What is novel and inventive in the present invention over the above prior art is the discovery that PEI bonds well to ordinary plastics surfaces and especially welled plastics plates and that such bonding is sufficiently stable to allow hybridisations to be carried out in the plates between the bound polynucleotide and its complementary partner.

Description of the preferred embodiments

The PEI used can be any possessing primary or secondary amino groups and cationic protonated amino groups, at a reasonably high molecular density. This will ordinarily require that the main chain or backbone of the polymer contains secondary or tertiary amino groups, and that there should be branching. Sufficient primary and/or secondary amino groups are required for good reaction with the cross-linking agent, e.g. as is well known in the immobilisation of proteins. Secondary amino groups can usually be cross-linked except when they are sterically hindered or the amine-cross-linking agent reaction is difficult. Sufficient protonated amino groups are required for attraction to DNA phosphate groups at many places in the molecule. These can be primary, secondary or tertiary amino groups, but bulky N-substituents which might hinder ease of approach to the DNA by the PEI are best avoided. With these principles in mind, it is suggested that at least 25%, preferably at least 50%, of the amino groups should be protonated and at least 5%, preferably at least 15% should be unprotonated primary amino groups.

Polyethylenimines of any of the kinds described by D. Horn, supra, and complying with the basic requirements of having primary or cross-linkable secondary amino and protonated amino groups are preferred. One particularly suitable PEI is "Polymin G-35" of BASF, the properties of which are described in a technical bulletin dated January 1981 available from BASF. It has a refractive index at 20°C of 1.445-1.455, a viscosity at 20° (Höppler) of 400-500 mPa.s, density of 1.06-1.12 g/cm^3 and a pH in 1% aqueous solution of 9.5-11.5.

From experiments it would appear that 50 micrograms of PEI is sufficient to coat each well. Higher levels, up to 500 micrograms per well, do not increase significantly the binding capacity of the plates. Concentrations of PEI below 50 micrograms per well reduce the binding capacity of the plates.

The welled plates include any of those currently available, which are broadly describable as of polyvinyl chloride or polystyrene. Other plastics will be found usable, including particularly copolymers of vinyl chloride or styrene with other monomers and acrylic polymers. Preferably microtitre plates having 96-wells are employed, these being stock equipment in most biological and clinical laboratories.

It is conceived that the invention will be operable in relation to any rigid plastics surface, although polystyrene and PVC are preferred. Interestingly, the PEI binds even to plates which are given a small positive electrical charge by the manufacturer.

The PEI-coated plates can be stored, e.g. for two months at 4°C, without loss of activity.

The PEI has to be linked durably to the polynucleotide which will require covalent bonding of some kind. It is believed that glutaraldehyde functions by bridging free primary or secondary amino groups on the PEI to free amino groups on the DNA bases, especially to amino groups in adenine, guanine and cytosine residues. Other NH_2-NH_2 linking groups or spacer arms known in

protein chemistry can be substituted, for example polyacrylic acid. Other kinds of cross-linkage to DNA are not excluded as a matter of principle. It appears that the glutaraldehyde also assists bonding of the PEI to the plastics surface.

The invention is expected to find most applicability in assays. The polynucleotide in the sample, herein referred to "analyte polynucleotide", can be any RNA or DNA or derivative thereof and can be of any length, within reason. It is normally wise to denature the polynucleotide to remove any undesired secondary structure or double-stranded material, thereby to minimise possible interference with hybridisation. Denaturing inevitably breaks down the length of the nucleic acid, typically to lengths of less than 500 nucleotides long. In certain circumstances, it might be wished to immobilise a single-stranded oligonucleotide, for example one synthesised chemically, as short as say 5 nucleotides in length, for example as a primer to enable a complementary DNA to be made in the wells of the plate by priming a nucleic acid in a sample.

The wells of most plates will bind a maximum of from 2 to 5 micrograms of polynucleotide depending on the amount of PEI coated, the length of the polynucleotide and the size of the well. Such large amounts of polynucleotide are not ordinarily required, however. The invention enables as little as 30 picograms of a particular polynucleotide in a mixture of polynucleotides to be detected, using biotinylated polynucleotide probes and enzyme-labelled streptavidin. When a radiolabelled polynucleotide probe is used the amount of bound radioactivity can be measured by cutting the wells from the plate. The concentration of polynucleotide can be adjusted appropriately to ensure that a suitable amount, normally between about 20 picograms and 5 micrograms, preferably between 1 nanogram and 1 microgram, is bound to the well.

The probe polynucleotide is complementary to the analyte polynucleotide in that it will hybridise to it under reasonable conditions. The probe can be labelled in any convenient way, e.g. with a radiolabel such as alpha ^{32}P, biotinylated, or enzyme-labelled by other means including the method of M. Renz et al., supra. The label can be "read out" by any appropriate method such as autoradiography, enzyme-labelled avidin or streptavidin (making use of the biotin-avidin or biotin-streptavidin interaction) or the use of an enzyme in the Renz et al. method. Another method of enzyme labelling a probe is described in PCT Application WO 84/03520 (Alan Malcolm et al.). The enzyme can be detected by adding an appropriate colour-forming, fluorescent or luminescent substrate and reading the colour, fluorescence or luminescence, as is well known in the immunoassay art.

The invention provides a valuable advance in the assay field by enabling assays for DNA to be carried out in multiwell plates. Other uses, such as in DNA sequencing, will suggest themselves to the expert in the art.

The following Examples illustrate the invention.

EXAMPLE 1

In this Example, polyvinyl chloride 96-well microtitre plates sold under the Trade Mark "FALCON" (Becton Dickinson Labware, California, USA) were coated with polyethylenimine (PEI). PEI "G-35" (BASF) was diluted to 10 mg/ml in 5 mM sodium phosphate buffer, pH 6.8. 100 microlitres of the diluted PEI were added to each well and the plates were incubated overnight at 4°C. The plates were then washed thoroughly with 5 mM sodium phosphate buffer, pH 6.8, and dried at room temperature and under reduced pressure. The dried plates were stored at 4°C.

The nucleic acid to be assayed (the "analyte" nucleic acid) was cloned double-stranded complementary DNA to foot and mouth disease virus RNA of type A10 strain 61 (see UK Patent 2084583B).

The nucleic acid was diluted with the above-mentioned phosphate buffer in order to avoid coating the plate with excess. The binding

capacity of the wells is about 5 micrograms of nucleic acid. A typically suitable concentration is one which provides 30 picograms to 5 micrograms per well. The DNA was then denatured and made single-stranded by immersing it in boiling water for 5 to 10 minutes and cooling it rapidly in an ice bath. 50 microlitres of the ss DNA solution was added to each well. 6 microlitres of 5 g/100 ml solution of glutaraldehyde in the above-mentioned phosphate buffer were added to each well. The plates were incubated at 37°C for 30 minutes with constant shaking and then washed thoroughly with the same phosphate buffer. The plates having DNA immobilised thereon were used for hybridisation.

For hybridisation, the same cloned FMDV and DNA was used. It was made single-stranded as described above and then nick-translated to replace deoxythymidine residues by biotinylated deoxyuracil residues, using a nick-translation kit supplied by BRL, Cambridge, UK. This biotin labelling method is that pioneered by D.C. Ward of Yale University and described in European Patent Specification 63879A.

Biotin-labelled probe nucleic acid having a length of 50-200 nucleotides was stored at a concentration of approximately 40 micrograms/ml in 5 mM phosphate buffer pH 6.8 at 20°C. Immediately prior to hybridisation the probe nucleic acid was denatured by boiling (2 to 3 minutes) and diluted to the final concentration of between 100 and 100 ng/ml in hybridisation buffer. The composition of the hybridisation buffer was as follows:-

50% Deionised formamide (BDH Chemicals, deionised with Amberlite resin, also from BDH Chemicals);

5 X SSC;

50 mM Sodium phosphate buffer pH 6.8;

0.04% w/v each of bovine serum albumin, Ficoll (type 70, Sigma Chemical Company), Polyvinylpyrrolidone (BDH Chemicals);

500 micrograms Ribonucleic acid from Torula Yeast Type VI, Sigma Chemical Company).

50 microlitres of hybridisation buffer was added to each well of the plate. Hybridisation was carried out overnight at 37°C with continuous shaking.

Hybridisation buffer was removed from the wells and the plate washed with several changes (4 or 5 normally) of 2 X SSC. Each of these washes was only short, being used principally to remove the hybridisation buffer and probe nucleic acid which remained in solution. The plate was then washed twice (5 minutes each) with 2 X SSC at 37°C. Finally the plate was washed 3 times (10 minutes each) in 0.1 X SSC at 50°C.

The washing conditions described above can be adjusted to suit the level of nucleic acid homology required.

The biotin-labelled nucleic acid was detected by the use of a streptavidin-biotinylated horseradish peroxidase conjugate (Amersham International PLC). The plate was washed once with Phosphate Buffered Saline (PBS) and 50 microlitres of a 1 in 500 dilution of the horseradish peroxidase conjugate, in PBS containing 2% w/v "Marvel" (Cadbury's), added to each well. ("Marvel" acts very efficiently as a general blocking agent, and is considerably cheaper than the more commonly used bovine serum albumin). The plate was incubated at 37°C for 15 minutes with continuous shaking.

Excess horseradish peroxidase conjugate was removed by thoroughly washing the plate with PBS followed by two brief washes with PBS containing 0.01% v/v Triton-X-100 and finally several more washes with PBS.

Horseradish peroxidase was detected using a 0.04% w/v solution of o-phenylenediamine (OPD) in citrate buffer (pH 6.6) containing 0.015% v/v H_2O_2. 50 microlitres of the OPD solution was added to each well and the plate incubated at room temperature for approximately 15 minutes. The reaction was stopped by addition of 50 microlitres of 1.25 N H_2SO_4 and the adsorbance at 492 nm measured on a Flow Titertek microtitre plate recorder.

EXAMPLE 2

Example 1 was repeated using as the analyte nucleic acid single-stranded RNA from FMDV type A10 strain 61 and cloned cDNA to make the probe.

The procedure of Examples 1 and 2 was carried out in a single 96-well plate using various dilutions of the analyte nucleic acid, from 5 to 250 picograms and a blank (no analyte nucleic acid) in the 12 wells of row A (ds DNA) and row C (viral RNA). Controls (no coating of the wells) occupied rows E and G. Each experiment was carried out in duplicate, in 12 wells per row, row B being a repeat of row A, row D a repeat of row C etc. The results of Table 1, show a detection limit of 30 picograms for the RNA. (This difference may be explained by making allowance for the length of nucleic acid sequence in the probe that can hybridise to the analyte nucleic acid. Both the cloned insert and the plasmid vector are available for hybridisation to the anlyte DNA whereas only the insert will hybridise to the bound RNA.) The control wells and the blanks all gave similar results indicating no hybridisation.

EXAMPLE 3

Example 1 was repeated using as the analyte nucleic acid the ds viral DNA of the sheep pox virus capripox. Cloned cDNA was used to make the probe.

TABLE 1

Figures represent the mean optical density measured at 492 nm, of duplicate wells.

	Well number and amount of analyte DNA per well											
	1	2	3	4	5	6	7	8	9	10	11	12
	250 pg	200 pg	150 pg	100 pg	60 pg	50 pg	40 pg	30 pg	20 pg	10 pg	5 pg	Blank
A-B	0.611	0.721	0.586	0.800	0.713	0.690	0.515	0.370	0.279	0.271	0.272	0.280
C-D	0.572	0.560	0.463	0.365	0.331	0.350	0.289	0.260	0.245	0.241	0.239	0.256
E-F	0.268	0.267	0.267	0.267	0.277	0.282	0.281	0.279	0.274	0.271	0.270	0.274
G-H	0.243	0.231	0.243	0.239	0.244	0.250	0.248	0.248	0.242	0.237	0.238	0.255

Key

Row A-B : Cloned ds DNA of FMDV RNA type A10 strain 61.

Row C-D : Virus RNA of FMDV type A10 strain 61.

Row E-F : As for A-B but without PEI coating.

Row G-H : As for C-D but without PEI coating.

EXAMPLE 4

Example 1 was repeated using as the analyte nucleic acid double-stranded RNA of the blue-tongue virus, one of the reoviridae family. For the probe, cDNA was made by random priming and reverse transcriptase.

EXAMPLE 5

Example 3 was repeated using polystyrene 96-well microtitre plates sold under the Trade Mark "NUNC" (Intermed, Roskilde, Denmark).

EXAMPLE 6

This Example (PEI) compares poly-L-lysine and protamine sulphate (1) for binding nucleic acid to microtitre plates and (2) for carrying out assays involving hybridisation of the bound nucleic acid.

Preparation of polymer-coated plates

"Falcon" PVC plates were used in both experiments. PEI, poly-L-lysine and protamine sulphate were diluted to 1 mg per ml in 5 mM sodium phosphate buffer, pH 6.8. Volumes of 100 microlitres of diluted polymer were added to each well, and the plates were incubated overnight, washed and dried as in Example 1. Duplicate rows of each of the polymer coatings were prepared. The remaining two rows of each plate were left uncoated to act as a blank control.

EXPERIMENT 1 : Binding assay

In this experiment labelled and unlabelled DNA, both a cloned copy of the genome nucleic acid of Capripox virus, were allowed to compete for binding sites on the polymer-coated plates. Radioactive label (^{32}P) was introduced into the nucleic acid by nick-translation. The labelled nucleic acid was separated from the unincorporated nucleotides by gel filtration, concentration by ethanol precipitation and finally diluted to a concentration of approximately 100 nanograms per ml in 5 mM sodium phosphate buffer, pH 6.8. Various dilutions of the unlabelled nucleic acid were prepared in the buffer containing

the labelled DNA. The final amounts of the unlabelled DNA from 0.5 nanogram to 10 micrograms/wells are shown in Table 2. The required dilutions were calculated by taking 50 microlitres as the volume required in each well.

The nucleic acid was denatured by boiling and a 50 microlitre volume of each dilution added to the rows of two polymer-coated plates. Glutaraldehyde (6 microlitres of a 5% w/v solution) was added to the wells of one of the plates and an equal volume of sodium phosphate buffer was added to the wells of the second plate. The plates were incubated at 37°C for 30 minutes and the unbound nucleic acid removed by washing thoroughly with sodium phosphate buffer. Simple monitoring of the plates suggested that the amount of the nucleic acid bound at this stage of the experiment was the same in the presence and absence of glutaraldehyde. Monitoring at this stage was by wide aperture geiger counter, sufficient to provide only a rough approximation of the radioactivity bound to the plates.

Both plates were treated using the conditions of hybridisation and washing described in Example 1, but no hybridisation was actually carried out. After the final wash the plates were dried and the amount of radioactivity within the individual wells measured by scintillation counting. The results are given in Table 2, in which the figures represent the mean number of counts from duplicate wells. The positive and negative symbols indicate the use or not respectively of glutaraldhyde to covalently link the DNA.

As Table 2 shows, both PEI and poly-L-lysine were highly effective in binding the DNA. The amount of unlabelled DNA which begins to compete with the labelled DNA for the polymer binding sites is 2.5 micrograms per well (56 microlitre volume) for both PEI and poly-L-lysine. The binding of DNA to both polymers was much reduced in the absence of glutaraldehyde. In contrast, protamine bound the nucleic acid poorly.

TABLE 2

Binding capacity of plates coated with polyethylene, poly-L-lysine and protamine sulphate, plus or minus glutaraldehyde

Amount of unlabelled DNA per well (56 microlitre volume)	Counts per minute per well							
	PEI		Poly-L-lysine		Protamine		Blank	
	-	+	-	+	-	+	-	+
10 µg	226	720	848	7505	672	697	62	403
5 µg	229	1980	940	11360	1650	1045	155	224
2.5 µg	288	20387	1833	14902	1150	2435	455	271
1 µg	494	28764	2564	14889	985	1907	475	296
50 ng	985	25531	5737	15263	1677	1175	405	131
25 ng	755	26528	1222	17412	1307	2079	295	367
10 ng	1258	24536	3801	15650	2107	2762	663	362
5 ng	840	27346	4871	22382	625	2319	379	439
2.5 ng	1650	28378	4295	18277	3687	2638	1804	443
0.5 ng	1888	24429	2137	19583	2027	2595	1453	282

EXPERIMENT 2 : Hybridisation assay

The results from this experiment are shown in Table 3.

Capripox virus DNA (whole genome DNA, not a cloned copy of the genome) was diluted in 5 mM sodium phosphate buffer, pH 6.8, to give final concentrations of between 200 nanograms per ml and 10 picograms per ml (10 ng/well to 0.5 pg/well respectively). The complete range of concentrations is shown in Table 3. The DNA was denatured by boiling and 50 microlitres of each dilution added to the wells of each row. Six microlitres of 5% glutaraldehyde was used to cross-link the DNA to the treated plate, thus giving a 56 microlitre volume in each well. The experiment was continued according to the method described in Example 1.

Cloned DNA from the capripox virus genome was used as the probe and labelled with biotin-dUTP by nick-translation. Hybridisation, washing, addition of streptavadin-biotinylated horseradish peroxidase, and assay for horseradish peroxidase was carried out exactly as described in Example 1.

The results show that of the three coating agents used in this experiment PEI was the most suitable for nucleic acid hybridisation. The limit of detection of the analyte DNA in the PEI coated wells was 100 picrograms. The decrease in optical density at the 5 ng and 10 ng/well concentrations is believed due to operator error.

Although the first experiment showed that poly-L-lysine was effective in binding DNA to the plates, the second experiment shows that the DNA is not available for hybridisation to the probe. There are several possible reasons for this observation. Either the poly-L-lysine is not a reliable agent for binding nucleic acid to plates, the nucleic acid is bound in a way that prevents hybridisation to the probe, or poly-L-lysine interferes with the hybridisation.

TABLE 3

The Figures represent the mean optical density, measured at 492 nm, of duplicate wells.

Row	Well number and amount of analyte DNA per well											
	1	2	3	4	5	6	7	8	9	10	11	12
	10 ng	5 ng	2 ng	1 ng	500 pg	200 pg	100 pg	10 pg	5 pg	2 pg	1 pg	0.5 pg
A-B	0.71	1.07	1.33	1.06	0.71	0.52	0.35	0.24	0.24	0.22	0.22	0.18
C-D	0.27	0.33	0.36	0.39	0.35	0.27	0.24	0.22	0.24	0;22	0.20	0.17
E-F	0.78	0.68	0.65	0.50	0.34	0.26	0.27	0.28	0.22	0.22	0.20	0.20
G-H	0.31	0.19	0.25	0.22	0.19	0.21	0.22	0.19	0.18	0.18	0.18	0.17

Key

Row A-B : PEI coated

Row C-D : Poly-L-lysine coated

Row E-F : Protamine sulphate coated

Row G-H : Uncoated 'blank' wells

Using protamine sulphate to coat the plates, the limit of detection of the analyte DNA was 500 picograms. Thus, it was less sensitive than PEI. However, it is possible that if relatively large amounts of a protamine salt are bound to the plate, it could be an alternative to PEI and accordingly this specification covers any such parallel aspect as part of the invention herein.

The following claims define some important aspects of the invention, but do not purport to include every conceivable aspect for which protection might be sought in subsequent continuing and foreign patent applications, and should not be construed as detracting from the generality of the inventive concepts hereinbefore described.

CLAIMS

1. A rigid plastics support having bonded thereto a branched polyethylenimine having (1) primary amino or cross-linkable secondary amino and (2) protonated amino groups.

2. A support according to Claim 1 in the form of a welled plastics plate having bonded to wells thereof a branched polyethylenimine having (1) primary amino or cross-linkable secondary amino and (2) protonated amino groups.

3. A support according to Claim 1 or 2 wherein the polymer is polyethylenimine is substantially as hereinbefore described.

4. A support according to Claim 1, 2 or 3 of polyvinyl chloride or polystyrene.

5. A method of immobilising a polynucleotide on a rigid plastics support which comprises incubating a support according to Claim 1, 2, 3 or 4 with the polynucleotide and with a cross-linking agent effective to link amino groups of the polyethylenimine to reactive groups on the polynucleotide.

6. A method according to Claim 5 wherein the polynucleotide is single-stranded.

7. A method of assay of a polynucleotide in an analyte, which comprises:

(1) if the polynucleotide is not already in a single-stranded form, free of secondary structure or duplex which would interfere with hybridisation, denaturing it to remove said secondary structure or duplex,

(2) immobilising the polynucleotide on a plastics support by a method according to Claim 5,

(3) probing the immobilised polynucleotide with a labelled complementary polynucleotide under hybridisation conditions, and

(4) detecting the presence or amount of label bound to the support.

8. A method according to Claim 7 wherein the polynucleotide is immobilised in wells of a welled plastics plate.